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Appraisal of state-of-the art

Animal models of inflammatory bowel disease

Abdo R. Jurjus[a,*], Naim N. Khoury[a], Jean-Marie Reimund[b]

[a]*Department of Human Morphology, Faculty of Medicine, American University of Beirut, Beirut, Lebanon*
[b]*Department of INSERM, Unité 381, rue Molière, Strasbourg, France*

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Abstract

In inflammatory bowel disease (IBD), experimental models have proven to be important tools for detecting potential therapeutic agents and for investigating the mechanisms of pathogenesis. This review is intended to cover recent advances in basic IBD model applications. The use of more than 20 animal models has allowed the detection of numerous protective pharmacological agents, including a number of immunomodulatory agents that have entered the therapeutic armamentarium.

The models have been classified into five main categories based on the methods of induction: gene knockout (KO), transgenic, chemical, adoptive transfer, and spontaneous (each with subcategories).

Keywords: Animal models; Crohn's disease; Inflammatory bowel disease; Ulcerative colitis; Therapeutic use of models; Methods

1. Introduction

Inflammatory bowel disease (IBD) is a common and chronic gastrointestinal disorder characterized by intestinal inflammation and mucosal tissue damage initiated and perpetuated by a dysregulated immune response along with several intra- and extraintestinal manifestations, including autoimmune phenomena. The purpose of this article is to review the various animal models available for use in IBD research and to evaluate the availability and use of these animal models in the testing of pharmacological molecules or agents that could lead to a possible cure for this devastating disease entity. Over the past two decades, a steadily increasing number of more than 20 experimental models with a variable range of clinical manifestations similar to those observed in human IBD have been developed. These models contributed greatly to important advances in our current understanding of the underlying mechanisms of inflammation and disease pathogenesis as well as treatment. Despite the varying nature of these models, the aspects that they have in common support greatly the concept that environmental factors affecting genetically susceptible hosts are responsible for the induction of IBD. However, the precise etiology of IBD, including Crohn's disease (CD) and ulcerative colitis (UC), remains unclear, although genetic, environmental, and immunologic influences may all contribute to the disease process.

In general, an appropriate or an optimal animal model should display certain key characteristics: the gut should exhibit morphological alterations, inflammation, symptoms and signs, pathophysiology, and course similar or identical to the human IBD. It is also recommended that the animal of interest should have a well-defined genetic background in addition to a well-characterized immune system, with reagents that are readily available and accessible for experimentation, along with well-defined criteria for successful management and/or manipulation. For the purpose of this review, animal models of IBD are categorized into five broad classes: (i) gene knockout (KO) models; (ii) transgenic mouse and rat models; (iii) spontaneous colitis models; (iv) inducible colitis models; and (v) adoptive transfer models. Each model will be discussed, taking into consideration the method of induction, the key characteristics, and the possible pathogenic mechanism in addition to its use in pharmacological trials.

* Corresponding author. Tel.: +961-3-308716; fax: +961-1-480687.
E-mail address: aj00@aub.edu.lb (A.R. Jurjus).

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2. IBD models

2.1. Gene knockout (KO) models

This category includes at least six different subcategories depending on the ways and means of induction.

2.1.1. Interleukin-2 KO/IL-2 receptor (R)α KO mice

Interleukin (IL)-2 is an important regulatory cytokine of the immune system with multiple functions. In 1993, Sadlack et al. (1993) reported that in mice with a disrupted IL-2 gene, approximately 50% would die between 4 and 9 weeks of age with splenomegaly, lymphadenopathy, and autoimmune hemolytic anemia. The rest of the animals developed chronic colitis between 6 and 15 weeks. The small intestine of these mice was intact, whereas the colon (from rectum to cecum) was severely affected with ulcers and wall thickening. Pathologically, crypt abscesses, mucin depletion, and dysplasia of the epithelial cells (which are the features of human IBD) were observed. In addition, infiltration of activated T cells and B cells with increased expression of MHC class II and increases in IgG1, IgE, and anticolon antibodies were also observed (Sadlack et al., 1993).

Boone et al. (2002) used the IL-2 KO model of UC to investigate the role that CD28 might have in defective apoptosis of intestinal lymphocytes. This approach was based on the fact that CD28 is a potent costimulatory molecule expressed on T cells that bind B7-1 or B7-2 on antigen-presenting cells (APCs) to enhance T cell proliferation, IL-2 production, increases expression of the antiapoptotic protein bcl-x_L, and prolongs lymphocyte survival (Boise, Minn, Noel, et al., 1995). Defective apoptosis may support the survival of pathogenic T cells, a chronically activated population of lymphocytes in IBD. They reported less activated T cells in CD28$^{-/-}$ intestines compared to wild-type mice, indicating that CD28 normally provides a functional costimulatory signal to gut lymphocytes. However, they proved that the total T cell number and level of T cell proliferation were unaffected by the reduced activation, and lack of Peyer's patches associated with CD28 deficiency. Thus, the proliferation of lamina propria T cells and homeostasis within the lamina propria T cell population were not dependent on CD28 costimulation. The authors concluded that CD28 costimulatory signals may be more important in supporting certain aspects of T cell activation than in supporting T cell proliferation and/or survival. When they combined the CD28 deficiency to the IL-2 deficiency (using the IL-2$^{-/-}$ CD28$^{-/-}$ model), they reported virtual absence of bcl-x_L expression together with the appearance of lymphadenopathy and colitis (Boone et al., 2002). Their work suggested that hyperexpression of bcl-x_L is not required for the inflammation seen in IL-2$^{-/-}$ and provided insight into the potential efficacy of immunotherapies directed at IBD in humans.

Another deficiency, that of β_2-microglobulin, was also studied together with the IL-2 deficiency in mice by Sohn et al. (2001). Investigations of mutations of the APC and p53 genes and microsatellite instability in colonic adenocarcinomas were carried out in this murine model genetically predisposed for development of chronic UC. It was found that the clinical features and molecular genetics, except for the mutational spectrum of the Apc gene, were similar to those of UC-associated colorectal cancer (CRC) in humans. Consequently, it was suggested that this model could be used for chemoprevention studies with agents, such as folate, short-chain fatty acids, and 5-aminosalicylic acid, that appear to be promising in the prevention of the development of UC-associated CRC (Eaden, Abrams, Ekbom, Jackson, & Mayberry, 2000; Sohn et al., 2001).

The IL-2 deficient mouse model of chronic colitis was also used by Varilek et al. (2001) to evaluate the anti-inflammatory role of green tea phyphenol (GrTP) extract. The authors tested whether GrTPs decrease disease activity, and they demonstrated that these inhibit inflammatory responses and might be useful in treating chronic inflammatory states. Explants of colon and lamina propria lymphocyte cultures were established from control and treated mice, and were supernatants collected. After 1 week, culture supernatant from GrTP-treated mice showed decreased spontaneous IFN-γ and tumor necrosis factor (TNF)-α secretion compared with that of controls. After 6 weeks, the GrTP group had less severe colitis as demonstrated by lower histologic scores and lower wet colon weights. These results were associated with lower plasma levels of serum amyloid A, increased weight gain, and improved hematocrits. These data therefore offered a promising indication that green tea and its polyphenol fraction may prove to be a useful dietary supplement in the treatment of chronic inflammatory diseases, such as IBD. However, the concentration (0.5% solution) used in the study is higher than that recommended and readily available in food sources (tea). Assuming that a cup (100 ml) of green tea contains 200–500 mg of GrTP, a 70-kg human would have to consume 100–200 cups of green tea to achieve an equivalent daily dose (Varilek et al., 2001). It is believed that further trials with lower doses need to be carried out, and the efficacy of the concentrated forms of green tea extracts that are being sold as natural supplements need to be evaluated.

2.1.2. IL-10 KO mice models

IL-10 is produced by T cells, B cells, macrophages, thymic cells, and keratinocytes. It down-regulates the function of T helper (Th)-1 cells, NK cells, and macrophages. In 1993, Kuhn, Lohler, Rennick, Rajewsky, and Muller (1993) reported that in IL-10$^{-/-}$ mice, inflammation occurred in the whole intestine. The lesions were observed mainly in the duodenum, proximal jejunum, and ascending colon. Pathological thickening of the intestinal wall, due to hyperplastic changes, was observed in the duodenum and jejunum. In the colon, goblet cell depletion, degeneration of the epithelium, infiltration of IgA-producing plasma cells, and an increase in MHC class II expression were detected. As in the IL-2$^{-/-}$

mice, the activation of $CD4^+$ Th1 cells and the depletion of their inhibitor, the regulatory T cells, are presumed to be the cause of the inflammation (Kuhn et al., 1993).

In 2000, Jijon et al. (2000) assessed the mechanism behind a poly (ADP-ribose) polymerase (PARP)-induced increase in epithelial permeability that is associated with chronic, nonresolving colitis, which develops spontaneously in the IL-10 gene-deficient mouse. They demonstrated that treatment of IL-10-deficient mice with PARP inhibitor 3-aminobenzamide reversed the typical signs of CD in these mice, namely, increased permeability, high levels of mucosal IFN-γ and TNF-α, and increased NO production (Jijon et al., 2000).

In the same year, Cantorna, Munsick, Bemiss, and Mahon (2000) used the IL-10-deficient mouse model to investigate the role of vitamin D on the course of IBD. The animals were divided into three groups: vitamin D deficient, vitamin D sufficient, and active vitamin D supplemented. In contrast with vitamin D-deficient IL-10 KO mice, vitamin D-sufficient mice did not develop diarrhea, waste, or die prematurely. To the authors' surprise, supplementation with active vitamin D for only 2 weeks blocked the progression and significantly ameliorated the symptoms in the mice that had established spontaneously developed IBD.

The importance of animal models for the evaluation of pharmacological strategies was further emphasized in a straightforward ministudy conducted by Gratz et al. (2002) who administered murine monoclonal anti-TNF-α antibodies intraperitoneally into IL-10 KO mice that had established IBD. Demonstration of significant histologic improvement of inflammation that correlated well with a resolution of diarrhea and rectal bleeding (Gratz et al., 2002) accentuated the pivotal role that TNF-α appears to play in the pathogenesis of IBD and emphasized the importance of this model.

Recently, a pioneer therapeutic approach to IBD was tested in the IL-10 KO model by Watanabe, Yamazaki, and Kanai (2003). The group developed poly-D,L-lactic acid microspheres containing dichloromethylene diphosphonate that, once administered rectally, were specifically taken up by macrophages, subsequently depleting them. The authors showed reduced numbers of resident macrophages in the intestinal lymphoid follicles in this model, associated with suppression of development of chronic colitis Watanabe, Ikuta, et al. (2003). This important finding invites further study, although depletion of gut macrophages might prove to be a controversial approach to IBD therapy.

The value of the IL-10 KO model was evaluated further by Lindsay, Ciesielski, Scheinin, Brennan, and Hodgson (2003), who induced IL-10 production in IL-10-deficient mice. Knowing that daily injections of IL-10 are unable to induce remission in mice with established disease (Lindsay et al., 2003), it was shown that rectal administration of adenoviral vectors encoding IL-10 (AdvmuIL-10) induced hepatic IL-10 release and lead to a long-term disease suppression with profound systemic immunoregulatory changes. This gene therapy using local delivery of AdvmuIL-10 reversed colitis and avoided the systemic effects seen after intravenous administration (Lindsay et al., 2003).

Furthermore, Rennick and Fort (2000) showed that IL-10 administered rectally to a small number of UC patients resulted in improved histological scores and diminished cytokine production by lamina propria and circulating mononuclear cells. However, long-term treatment with IL-10 needs to be fully assessed inasmuch as multiple cytokines are dysregulated in IBD patients, and those involved in perpetuating the chronic phase of disease may differ between individuals, as is the case in experimental animal models. Therefore, the most successful therapeutic strategies will probably include combinations of drug, cytokine-based (i.e., IL-10), and antibody-based (i.e., anti-TNF) treatments (Rennick and Fort, 2000).

2.1.3. T cell receptor (TCR) mutant mice

It was reported by Mombaerts et al. (1993) that colitis occurred in TCR mutant mice. At 16 weeks after birth, soft stools, consistent inflammation, and hypertrophy of the entire colon (from rectum to cecum) were observed in this model, but the small intestine remained intact. Hyperplasia of the colonic epithelium, a decrease in the number of crypt abscesses and goblet cells, and infiltration of lymphocytes, plasma cells, and neutrophils were also noted. B cells were polyclonally activated, and a number of autoantibodies were produced as a result of the immunological disorder. This pathology was regarded as a UC-like model, due to the distribution of the lesions, the pathological findings, and the fact that it has Th type-2 colitis. It was also reported that anti-IL-4 antibody inhibits the activation of colitis in $TCR^{-/-}$ mice, and a therapeutic effect of this antibody in human UC patients may be anticipated (Bhan, Mizoguchi, Smith, & Mizoguchi, 1999; Mombaerts et al., 1993; Takahashi, Kiyono, & Hamada, 1997).

2.1.4. TNF-3' untranslated region (UTR) KO mice

Some large-scale controlled studies in the United States and Europe have proven the efficacy of anti-TNF-α antibody (CA2) as a therapeutic drug (D'Haens et al., 1999; Hibi, Ogata, & Sakuraba, 2002; Targan et al., 1997). A transgenic mouse with overexpression of human TNF-α was introduced as an arthritis model, but no colitis was observed in this model. It was reported that colitis would occur by the overexpression of TNF-α in a different way. In the 3′-UTR area of TNF-α, there is an adenine/uracil-rich element (ARE) consisting of AUUUA repeats. An ARE also exists in the 3′-UTR area of IL-2, c-*fos*, and granulocyte macrophage–colony-stimulating factor (GM–CSF); it destabilizes the mRNA of the cytokines in the upstream region (Kontoyiannis, Pasparakis, Pizarro, Cominelli, & Kollias, 1999). Several findings, based on KO studies targeting TNFR-1, ARE of TNF-α and TNFR-2 suggested that in TNF-3′-UTR KO mice, TNFR-2 was inhibitory for arthritis and that

lymphocytes were essential for induction of colitis but not for arthritis (Kontoyiannis et al., 1999).

2.1.5. Trefoil factor-deficient mice

Intestinal trefoil factors (ITFs) are peptides secreted by mucus cells of the gastrointestinal tract after inflammatory damage. Mice with targeted disruption of ITF show severely impaired mucosal healing and decreased epithelial regeneration. They die after the induction of colitis by the addition of dextran sulfate sodium (DSS) to the drinking water. A beneficial role has been reported for ITF in repair processes within the intestinal mucosa in acetic acid or trinitrobenzene sulfonic acid (TNBS)-induced colitis in rats. Therefore, these mice could be useful in studying wound-healing processes in the gut and therapeutic approaches for intestinal injury (Mashimo, Wu, Podolsky, & Fishman, 1996).

2.2. Transgenic mouse and rat models

2.2.1. IL-7 transgenic mice

The epithelial cell-derived IL-7 was shown to be an essential cytokine for the proliferation and functional regulation mechanism of epithelial cells, intraepithelial lymphocytes, and intramucosal lymphocytes. It has been demonstrated that IL-7 was the substance within the serum of UC patients that influenced the differentiation and proliferation of T cells in the thymus (Hibi et al., 2002).

Further investigation of IL-7 transgenic mice, which overexpress IL-7 mRNA, revealed that acute colitis occurred in them at 1 to 3 weeks of age along with an infiltration of neutrophils, $CD4^+$ T cells, and $\gamma\delta$ T cells in the intestine. High levels of IL-7 protein expression were found in the inflamed regions of the intestine in these mice. At 8–12 weeks of age, proctoptosis with anal bleeding occurred. Pathologically, serial and diffuse infiltration of monocytes, decreases in goblet cells, and increases in crypt abscesses were observed in the lamina propria of the intestine. This is therefore a chronic colitis model that closely resembles human UC. In contrast to acute colitis, IL-7 protein was decreased in this chronic colitis model. This is presumed to be due to the decrease in goblet cells that are rich in IL-7. It is suggested that in the acute phase, the excessive secretion of IL-7 induces activation of mucosal lymphocytes which causes colitis, while in the chronic phase, apoptosis of the activated lymphocytes, which results from the lack of IL-7, is presumed to be the cause of colitis (Watanabe et al., 1997).

Recently, Watanabe, Yamazake, et al. (2003) have established the essential role of mucosa IL-7/IL-7R-dependent signals in the development of chronic intestinal inflammation. Their results indicated that mucosal IL-7/IL-7R-dependent signals are involved in the development of chronic intestinal inflammation in both the mouse model and human disease of the intestinal mucosa (Watanabe, Yamazake, et al., 2003). This animal model of colitis turned out to be of extreme importance inasmuch as it elucidated a major mechanism in the pathogenesis of IBD and gave an insight to a possible therapeutic approach.

2.2.2. Signal transducer and activating transcription (STAT)-4 transgenic mice

Recently, seven STAT molecule families, which are essential in the signal transduction of many cytokines, have been reported. Each member of a STAT family works for several cytokines, and this may be a reason for the redundancy of cytokines. STAT-4 is peculiar to the signal transduction of IL-12, and STAT-4 $^{-/-}$ mice are a representative KO model of Th type-1 colitis (Wirtz et al., 1999).

2.2.3. HLA B27 transgenic rats

Rats transgenic for human HLA-B27 (a molecule involved in human spondyloarthropathies) and β2-microglobulin develop a spontaneous IBD that affects the stomach, ileum, and the entire colon. Crypt hyperplasia and mucosal infiltration of mononuclear inflammatory cells mostly characterize the disease. A functional role of activated type-1 helper T-lymphocytes in the pathogenesis has been suggested. This model has been used extensively to study the effect of resident intestinal bacteria in the acute and chronic stages of gastrointestinal inflammation. These studies have demonstrated that various bacterial species can induce diverse types of pathology, for example, colitis and gastritis, in these rats (Rath et al., 1999). Rath et al. (2001) added ciprofloxacin, metronidazole, and vancomycin/imipenem in drinking water to the HLA B27 transgenic rats and found that (1) although preventive metronidazole significantly attenuated colitis, there was no benefit in treating with metronidazole once colitis was established; (2) antibiotic therapy with vancomycin/imipenem was effective in both treatment and prevention, although it did not completely abrogate colitis; and (3) ciproflaxin had similar histologic effects as metronizadole (Rath et al., 2001). Taking into consideration the pharmacological class of the antibiotics administered, Rath (2003) concluded that defined bacterial subsets initiate colitis, but a much broader range of commensal bacteria can provide the constant antigenic drive for chronic inflammation once mucosal permeability is altered.

2.3. Spontaneous colitis models

2.3.1. C3H/HejBir mice

C3H/HejBir is a derivative of selective breeding of C3H/Hej mice with colitis known to develop occasionally perianal ulcers and colitis. In C3H/HejBir mice, colitis is limited to ileocecal lesions and the right side of the colon. It occurs spontaneously in the third to fourth week of life and disappears after 10–12 weeks. Ulcers, crypt abscesses, and regeneration of epithelium are seen, but thickening of the intestinal wall and granulomas are not observed. Increased levels of IFN-γ and IL-2 have been detected in the lamina propria lymphocytes, which suggests that colitis in

this model is a Th type-1 response. This model has also been used in combination with inducible colitis models and has proven to be valuable for studying and identifying genetic susceptibility factors (Cong et al., 1998).

2.3.2. SAMP1/Yit mice

According to Riviera-Nieves et al. (2003), SAMP1/Yit mice spontaneously develop chronic terminal ileitis, reminiscent of the human disease described by Crohn et al. in 1932. Several new phenotypic features have appeared in their colony after more than 20 generations of brother–sister mating. In their report, they described the distinguishing features of the University of Virginia SAMP1/YitFc substrain, compared with the Japanese SAMP1/Yit parental strain (Matsumoto et al., 1998). The following differences were observed: (1) SAMP1/YitFc mice displayed established ileitis as early as 10 weeks of age; (2) the incidence of skin lesions inversely correlated with the occurrence of intestinal inflammation; (3) mice developed chronic ileitis with prominent muscular hypertrophy and focal collagen deposition in inflamed segments; (4) mesenteric lymph node lymphocytes acquired an activated phenotype coincident with disease progression; (5) high IFN-γ production was detected by 4 weeks of age and preceded the onset of ileitis; (6) finally, a subgroup of mice (approximately 5%) developed perianal disease with ulceration and fistulae. They concluded that the SAMP1/YitFc substrain exhibits unique characteristics when compared with the original Japanese strain. Of particular interest was the emergence of perianal fistulizing disease, which was the first report of such occurrence in an animal model of IBD (Riviera-Nieves et al., 2003).

Marini et al. (2003) have reported that administration of anti-TNF-α antibody had a similar therapeutic effect to that of infliximab, a chimeric monoclonal antibody against human anti-TNF-α. The similarities between the observed effects of anti-TNF-α in the murine model with those obtained in patients with CD suggested similar mechanisms of action of TNF-α blockade. Further analysis proved that this treatment decreased the percentage of apoptotic cells in freshly isolated intestinal epithelial cells, an observation that was associated with an increase in the percentage of apoptotic lamina propria mononuclear cells. This study paved the way for the development of novel therapeutic approaches, mainly targeting intestinal cells apoptosis.

Another pharmacological trial was carried out in this model of spontaneous colitis by Bamias et al. (2002). The intestinal Th1 cytokine production characteristic of this model and activation of gut lymphocytes were found to be significantly inhibited and down-regulated, respectively, by ciprofloxacin and metronidazole antibiotic therapy in both prevention and treatment protocols. This antibiotic approach decreased significantly the severity of ileitis both in the prevention (40% reduction) and the treatment (25% reduction) protocols, compared with untreated control mice (Bamias et al., 2002).

2.4. Inducible colitis models

2.4.1. Acetic-acid-induced colitis

Epithelial or mucosal necrosis and transient inflammation can be induced by luminal instillation of dilute acetic acid in a dose-responsive fashion (Elson, Sartor, Tennyson, & Riddell, 1995). In the original description of the model (MacPherson & Pfeiffer, 1978), 0.5 ml of 10–50% acetic acid diluted with water was instilled into the rectum of Sprague–Dawley rats. After 10 s of surface contact, the acidic solution was withdrawn, and the lumen was flushed three times with 0.5 ml saline. In a later modification (Yamada, Marshall, Specian, & Grisham, 1991), 1 ml 4% acetic acid (pH 2.3) was slowly infused 5 cm into the rectal lumen of a lightly anesthetized rat. After a 30-s exposure, excess fluid was withdrawn, and the colon was flushed with 1.5 ml PBS. Many further modifications have been introduced throughout the years, and most subsequent studies have used 15- to 30-s exposures to 4% or 5% acetic acid in both enema and ascending colon models because higher concentrations induced frequent perforations (Elson et al., 1995). The initial injury in this model was a relatively bland epithelial necrosis and edema that variably extended into the lamina propria, submucosa, or external muscle layers, depending of the concentrations and length of exposure of acetic acid. Epithelial injuries were a relatively specific reaction to organic acids because HCl at similar pH did not induce a similar injury (Yamada et al., 1991). Transient local ischemia might contribute to the acute injury, but neutrophils were apparently not involved in very early phases. Mucosa and submucosal inflammation followed initial injury and was associated with activation of arachidonic acid pathways (Elson et al., 1995). Acetic-acid-induced colitis is an easily inducible model of IBD, and the similarity of the inflammatory mediators profile to IBD suggest that the inflammatory phase bears some resemblance to acute human intestinal inflammation (Elson et al., 1995).

Treatment with either antioxidant (Choudhary et al., 2001) or antianginal (Kuralay et al., 2003) drugs improved the macroscopic and microscopic scores of this model.

2.4.2. Iodoacetamide-induced colitis

This model, initially described by Satoh, Sato, Takami, and Szabo (1997), is based on the fact that endogenous sulfhydryl (SH) compounds, such as glutathione, play an important role in the protection of gastric mucosa. Instillation of SH blocker in the colon could induce colitis and cause injury to the mucosa by decreasing the amount of defensive SH compounds. After the induction of UC, many changes characteristic of inflammation were taken into account to determine the degree or severity of the disease related to the administered doses of iodoacetamide. The different alterations included diarrhea, dilatation, adhesion, mucosal damage (varying from slight mucosal erosion to deep lesion), and inhibition of body weight gain (Satoh et al., 1997).

Heparin, a polyanionic highly sulfated linear polysaccharaide, belonging to the family of glycosaminoglycans, has been implicated in the management of UC. Heparin therapy resulted in significant improvement in macroscopic and microscopic features of colitis, accompanied by a partial reduction in myeloperoxidase (MPO) levels. The regular trends in the behaviour of colonic fibroblast growth factor (FGF)-binding activity and HB–epidermal growth factor (EGF)-binding activity levels were reversed with heparin therapy (Levine et al., 2002). However, conflicting studies and results have been published on the response to heparin in patients with severe UC since it was first reported (Levine et al., 2002). The investigators concluded that one mechanism of heparin-mediated improvement in colitis may involve tissue healing associated with changes in functional levels of colonic growth factors. On the other hand, using the same model, it was shown that anti-surfactant-like particles antibodies ameliorated the inflammation in IBD (Higgins, Frankel, Douce, Dougan, & MacDonald, 1999).

2.4.3. Indomethacin-induced enterocolitis

It was shown that indomethacin induces small intestinal and colonic ulceration in a dose-dependent fashion in rodents (Elson et al., 1995). Reproducibility of this model is dependent on complete solubilization of indomethacin (in 100% alcohol and then diluted with 5% sodium bicarbonate or in methyl cellulose) and administration in the regular diet because fasted rats develop attenuated lesions. Initial epithelial damage is mediated partly by synthesis inhibition of the protective prostaglandins PGE1, PGE2, and prostacyclin. Luminal bacteria and bacterial products clearly contribute to the inflammatory response. This model has the advantage of being easily induced, in acute or chronic phases. It involves small and large intestines and is associated with extraintestinal lesions (Elson et al., 1995). Although small bowel ulceration and transmural inflammation have some similarity to CD, the chronic ulcerations are located in the mid-small intestine rather than the ileum.

Using the indomethacin-induced colitis model, Krieglstein, Anthoni, et al. (2001) examined the effect of the *Boswellia* extract and its single constituent acetyl-11-keto-β-boswellic acid (AKBA) on leukocyte–endothelial cell interactions in experimental colitis. Following oral treatment with *Boswellia* or AKBA, the increased leukocyte–endothelial cell adhesive interactions and severe tissue injury that accompanied indomethacin-induced ileitis were reduced in a dose-related fashion, with a decrease in rolling (up to 90%) and adherence (up to 98%). Injury scores were also reduced following this therapy indicating the anti-inflammatory actions of *Boswellia* extract in IBD, which may be due in part to boswellic acids such as AKBA (Krieglstein, Anthoni, et al., 2001).

2.4.4. TNBS-induced colitis

Colitis would occur in mice by treatment with a TNBS enema after destruction of the mucosal barrier with an ethanol enema (Hibi et al., 2002). Susceptibility to TNBS varied in each mouse, but some developed hapten-induced delayed-type hypersensitivity and proceeded to develop chronic colitis. Granulomas with infiltration of inflammatory cells in all layers were seen in the intestine of this model. The isolated macrophages produced large amounts of IL-12, and the lymphocytes produced large amounts of IFN-γ and IL-2. This evidence suggested that the colitis seen in this model was induced by a Th type-1 response, constituting a CD model (Neurath, Fuss, Kelsall, Stuber, & Strober, 1995).

A study by Stein, Ries, and Barret (1998) using TNBS-induced colitis shed light on intestinal epithelial barrier function and the possible role of mast cells. They noted that water absorption in the inflamed mucosa was markedly diminished. This effect would be expected to contribute to the diarrhea that occurs not only in this animal model but also in human IBD. Stein et al. reported evidence showing that alterations in epithelial function may be produced, either directly or indirectly, by products released from activated mast cells.

The similarities between the human disease and TNBS-induced colitis allowed Ilan et al. (2000) to address the immune system involvement in the pathogenesis of IBD, in particular, oral tolerance induction as new therapeutic grounds have the potential to be used in the clinical practice. They demonstrated that oral administration of colitis-extracted proteins induced immune tolerance, down-regulated the inflammatory immune response, and alleviated the colitis. Accordingly, IBD could be considered as an imbalance between proinflammatory and anti-inflammatory mediators. Adoptive transfer of oral tolerance using low-dose antigen feeding extracted from diseased bowel changed the proinflammatory/anti-inflammatory cytokine balance by shifting the immune response from a Th1 to a Th3 type, with marked alleviation of clinical, macroscopic, and microscopic manifestations of IBD (Ilan et al., 2000).

A study by Camoglio et al. (2002) questioned the common immunomodulation therapy in CD using anti-IL-12p40 antibodies. Their data showed that IL-12p40 and IL-12p35 differentially regulated the development of colitis with a protective role for IL-12p40. IL-12 is a heterodimer (p70) composed of two disulfide linked subunits, p35 and p40, which are both essential for IL-12 function. Using the TNBS-induced colitis model, they searched for the factor that was responsible for the induction of colitis in IL-12p35$^{-/-}$ KO mice. They showed that IL-12 was dispensable for IFN-γ responses after TNBS treatment and suggested that INF-γ was not responsible for TNBS-colitis as shown previously by other studies. Inasmuch as the TNBS model was regularly used in an attempt to reproduce human CD in animals, Camoglio et al. concluded that inhibition of IL-12p35 and IL-12 receptor signaling by specific drugs might be more promising than other common therapeutic practices in the prevention of relapsing CD.

The TNBS model served in clinical investigations for the development and testing of therapeutic molecules that have

the potential to be used in the clinic for the management of the human disease. To mention a few of these studies, Kankuri et al. (2001) reported Nimesulide reduction of the formation of inflammatory edema, probably by a mechanism related to inhibition of PGE2 production by COX-2 pathway, whereas Mane et al. (2001) determined the effect of L-Arg on the degree of intestinal inflammatory damage and the subsequent tissue repair. In addition to that, McCartney, Ballinger, Vojnovic, Farthing, and Warner (2002) showed that two nonselective endothelin $(ET)_{A/B}$ receptor antagonists, bosentan and Ro 48-5695, ameliorate the progression of tissue damage, particularly if administered prior to the induction of colitis in TNBS models. On the other hand, recent investigations by El-Haj, Poole, Farthing, and Ballinger (2002) established that central IL-1 receptors mediated the increase in activity of hypothalamic serotonin (5-HT) neurons from the hypothalamic paraventricular nucleus (PVN), the food control center in the hypothalamus, associated with TNBS colitis. This evidence supported previous conclusions that hypothalamic 5-HT contributed to anorexia but was not the only mediator (Ballinger et al., 2000).

2.4.5. Oxazolone colitis

Boirivant, Fuss, Chu, and Strober (1998) reported that an enema of oxazolone with ethanol would induce colitis. In comparison with TNBS, this agent caused colitis earlier. The peak of body weight loss and diarrhea was seen on the second day after the enema, and symptoms diminished after 10–12 days. Colitis, accompanied by ulcers, was localized in the distal colon. Histopathological studies showed that the numbers of epithelial cells, goblet cells, and glands have decreased compared with controls. In contrast to TNBS colitis, these findings closely resembled those of UC (Boirivant et al., 1998).

2.4.6. DSS colitis

The administration of DSS dissolved in water to mice or rats caused hematochezia, body weight loss, shortening of the intestine, mucosal ulcers, and infiltration of neutrophils. Acute colitis, which occurred during the administration of DSS, and chronic colitis, which occurred a little time after the administration of DSS, were seen in this model. Acute colitis was considered to be induced by innate immunity but not acquired immunity because it also occurred in severe combined immunodeficiency (SCID) mice. However, chronic colitis was considered to be caused by lymphocytes that are activated by the cytokines secreted from the activated macrophages (Okayasu et al., 1990).

In 1998, Shintani et al. (1998) used the DSS model to obtain more direct evidence for the involvement of T cells in the development of DSS-induced colitis. They performed adoptive transfer experiments in which colitis-derived T cells were primed by cultivation with DSS-pulsed macrophages in vitro and then transferred to normal mice. Their data clarified the involvement of $CD4^+$ T cells in the pathogenesis of DSS-induced colitis. In addition, they demonstrated that IgG inhibited the proliferation of pathogenic T cells by interaction with the cells in vitro. These results suggested that IgG may modulate the development of colitis through the attenuation of T cell proliferation, leaving the exact mechanisms of IgG at call for examination in the future (Shintani et al., 1998).

Marrero, Matkowskyj, Yung, Hecht, and Benya (2000) used the DSS model of murine colitis to determine whether Gal1-R expression was up-regulated in association with colonic inflammation and to evaluate the ability of galanin to increase colonic fluid secretion. It was shown that DSS caused a progressive and severe colitis and that the worsening colitis was associated with increased activation of NF-êB, which in turn paralleled the increases observed in Gal1-R expression (Marrero et al., 2000). Their data supported the possibility that increased Gal1-R expression may be an important component of the excessive fluid secretion observed in IBD. Although their study did not attempt to validate the use of the DSS model for the study of IBD, it directly associated galanin and Gal1-R with the pathophysiology of IBD-associated diarrhea.

Other workers, using the DSS model in BALB/c mice, tested in a series of experiments the effect of type-IV phosphodiesterase inhibitors. They documented the efficacy of rolipram and mesopram, two potent suppressors of TNF-α synthesis, in both the prevention and treatment of experimental colitis similar to CD (Hartmann et al., 2000; Loher et al., 2003).

Inasmuch as a key role in the pathogenesis of human UC and CD has been attributed to molecules that regulate the recruitment of leukocytes, such as cell adhesion molecules (CAMs), Soriano et al. (2000) investigated the involvement of three of these molecules in a DSS model of murine colitis. They studied the intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and mucosal addressing cell adhesion molecule 1 (MAdCAM-1), which are endothelial CAMs of the immunoglobulin superfamily with a critical role in mediating the firm adhesion of leukocytes to endothelial cells in a variety of acute and chronic inflammatory diseases. Comparison of the therapeutic value of selective immunoneutralization of ICAM-1, VCAM-1, or MAdCAM-1 showed clearly that VCAM-1 blockade resulted in a significant amelioration of colitis, superior to that of ICAM-1 or MAdCAM-1 blockade (Soriano et al., 2000). This work represented a novel approach for the treatment of IBD conditions.

Another study by Kato et al. (2000) addressed the involvement of MAdCAM-1 in IBD using the same mouse model used by Soriano et al. (2000). The results on the prophylactic effect of anti-MAdCAM-1 antibody treatment on DSS-induced colitis led Kato et al. to suggest that MAdCAM-1 might be a specific therapeutic target for colitis and accordingly concluded that anti-MAdCAM-1 therapy might be an effective and organ-specific strategy for treating human IBD (Kato et al., 2000).

The expression of many inflammatory cytokines genes (IL-1, TNF-α, IL-6, and IL-8) and some adhesion molecules genes [endothelial leucocyte adhesion molecule-1 (ELAM-1) and ICAM-1] are both regulated by the transcription factor, nuclear factor κB (NF-κB). In the course of colonic inflammation, such as in IBD, colonic mucosa shows increased expression of these molecules. Common therapeutic approaches to human IBD include the administration of either sulfasalazine or corticosteroids which have very often serious side effects. In view of these undesirable effects of traditional treatment, intracolonically administered NF-κB (p65) antisense oligonucleotide was tested by Murano et al. (2000) in DSS-induced colitic mice in an attempt to alleviate inflammation seen in this animal model of UC. This procedure has also been successfully conducted before on TNBS-induced colitis (Neurath, Pettersson, Meyer zum Buschenfelde, & Strober, 1996). In their study, Murano et al. showed the effectiveness of NF-κB (p65) antisense oligonucleotides on rectal inflammation when administered on Day 0 or 2. However, no effect was observed when antisense oligonucleotides were administered on Day 7. These results suggested that NF-κB antisense oligonucleotides have inhibitory effects on DSS-induced colitis when administered in the early phase of inflammation. Administration of NF-κB antisense oligonucleotides down-regulated effectively the production of the abovementioned inflammatory cytokines in the colonic mucosa. The decreased expression of p65 was monitored by Western blotting analysis to show the direct effect of antisense oligonucleotides (Murano et al., 2000).

Taking into consideration the possible influence of fecal bile acids on the clinical course of IBD, Araki et al. (2000) assessed the eliminating effects of fecal bile acids by the oral adsorbent on DSS-induced colitis in rats and found that oral adsorbent tended to attenuate the disease and promoted the recovery process. Thus, their results confirmed the involvement of bile acids in IBD and suggested that oral therapies with localized pharmacological properties are potent therapeutic approaches to IBD. However, elimination of bile acids in the gut lumen, although proved to limit the progression of DSS colitis and positively modulate its repair process, must be delicately evaluated before clinical trials are initiated considering the important physiologic properties of bile acids in the gut (Araki et al., 2000).

On the other hand, Krieglstein, Cerwinka, et al. (2001) addressed the implication of reactive oxygen and nitrogen species (RONS) in the pathogenesis of IBD using the DSS model of colitis. The two most extensively studied RONS, superoxide (O_2^-) and nitric oxide (NO), are known to exert profound (and often opposing) effects on inflamed tissue. Krieglstein, Cerwinka, et al. used gene-targeted animals to assess the contributions of three key RONS-related enzymes [iNOS, superoxide dismutase (SOD), and NADPH oxidase] to the gut inflammation and tissue injury in DSS-induced colitic mice. Their findings indicated that either the genetic absence or pharmacologic blockade of iNOS significantly attenuated the severity of colonic inflammation. On the other hand, mice that genetically overexpress CuZn-SOD exhibited exaggerated inflammatory and tissue injury responses to DSS treatment. The absence of functional NADPH oxidase, resulting from targeted disruption of p47phox, did not alter susceptibility to DSS-induced intestinal inflammation, provided that iNOS remained functional. Using the specific iNOS inhibitor 1400W, they demonstrated that the combined blockade of iNOS (by 1400W) and NADPH oxidase (by genetic deletionof p47phox) was even more effective in protecting mice from DSS colitis than either intervention alone. These results suggested that both iNOS and CuZn-SOD played an important role in the progression of DSS-induced colitis. NADPH oxidase per se might only play a minor role in the presence of functional iNOS inasmuch as genetic blockade of this enzyme alone did not influence the course of colitis. Such experiments allowed an in-depth look at the mechanisms involved in the pathogenesis of a widely used model of murine intestinal inflammation.

Using the DSS model for experimental colitis, Ahn et al. (2001) demonstrated that colonoscopic examination of experimental rats for the monitoring of the disease was as efficient as other examination procedures. They clearly showed that the results of noninvasive colonoscopic assessment of the severity of colitis were well correlated with the gross or pathological changes and that they were useful in predicting the efficacy of drug intervention. Ahn et al. evaluated the efficacy of drug intervention colonoscopically by treating experimental rats with an antioxidant substance, DA-9601, an extract of *Artemisia asiatica* known to possess antioxidative and cytoprotective actions and to be effective in ethanol or nonsteroidal anti-inflammatory-drug-induced gastric mucosal injury, cerulein-induced pancreatitis, or trinitrobenzoic-acid-induced colitis. As an anti-inflammatory drug, they selected sulfasalazine, a well-known drug possessing part of antioxidative actions in addition to an anti-inflammatory effect. Along with the important value of colonoscopy in experimental animals demonstrated in these experiments, Ahn et al. showed that antioxidant treatment is equally as or more effective than current anti-inflammatory drugs, either in decreasing the potential damaging factors, such as oxygen-derived free radicals or in decreasing colonic neutrophilic infiltrations (Ahn et al., 2001).

Lately, much research has focused on the search for antioxidants that have a preventive action against development of CRC in chronic colitis. The DSS model of IBD was adopted by Seril, Liao, Ho, Warsi, et al. (2002) to study the effect of the antioxidant *N*-acetylcysteine (NAC) on UC-associated cancer development. Their data indicated that NAC has the potential to serve as a preventive agent, possibly via inhibition of cellular proliferation and nitrosative stress-caused cellular damage. In fact, NAC significantly reduced tumor incidence in the DSS mice as well as the tumor multiplicity. The tumor volume was lower but not significantly decreased, whereas the proliferation index was

significantly decreased in noncancerous epithelia but not in tumor cells. Between cancer prevention and cancer treatment, NAC seemed to be more potent in the former. This animal model of chronic colitis proved to be useful to demonstrate how the efficacy of a pharmacological molecule could be tested either for prevention or for treatment of UC-associated CRC (Seril, Liao, Ho, Warsi, et al., 2002).

Nitrosative stress-caused cellular damage as well as augmented oxidative stress were also investigated by Seril, Liao, Ho, Yang, et al. (2002) in another study that focused on dietary iron supplementation in the management of human UC. They were able to demonstrate, using the DSS model of colitis, a marked increase in iron deposition on the epithelial surface of the colon and in the inflamed areas. In a long-term carcinogenesis experiment, a twofold iron-enriched diet significantly increased colorectal tumor incidence (88%) as compared with animals that were fed with the control diet (19%). Inasmuch as chronic UC patients frequently require iron supplementation to remedy anemia due to blood loss, the effect of iron supplementation on UC-associated carcinogenesis should be taken into consideration (Seril, Liao, Ho, Yang et al., 2002).

2.4.7. Peptidoglycan–polysaccharide (PG–PS) colitis

In 1988, Sartor, Bond, and Schwab (1988) demonstrated that the intramural injection of the bacterial cell wall component PG–PS into the distal colon of rats induced transmural enterocolitis. In genetically susceptible Lewis rats, chronic granulomatous colitis developed 3–4 weeks after injection. Histopathologically, there were thickening of the colon wall, infiltration of lymphocytes, macrophages, and neutrophils. PG–PS increased mucosal permeability and MPO activity, and enhanced NO production and collagen synthesis. Data obtained from this model clearly showed that the cell wall components of nonpathogenic resident enteric bacteria are sufficient to induce acute and chronic colitis in a susceptible host when they penetrate the colon wall (Sartor et al., 1988).

2.5. Adoptive transfer models

2.5.1. Colitis induced by transfer of heat shock protein (hsp) 60-specific CD8 T cells

Severe, generally lethal intestinal pathology, predominantly in the small intestine, was induced in this model by the adoptive transfer of an hsp60-specific $CD8^+$ T-lymphocyte clone preactivated by bacterial hsp60, into $TCR^{-/-}$ or SCID mice. The formation of colitis in these mice required the presentation of hsp60 on MHC class I and depended on a functional role of TNF-α. In contrast to the findings obtained in many other models, intestinal inflammation in this model does not depend on the presence of the resident bacterial flora. Thus, the results obtained by the initial analysis of this model indicated that autoimmune hsp60 $CD8^+$ T cells that were reactive to cellular hsp60 mediated the pathogenesis of colitis (Steinhoff et al., 1999).

2.5.2. CD45RB transfer model.

As shown by Morrissey, Charrier, Braddy, Liggitt, and Wason (1993), the adoptive transfer of $CD4^+$ T cells expressing high levels of the surface molecule CD45RB ($CD4^+$ $CD45RB^{hi}$) into SCID recipients (i.e., $CD4^+$ $CD45RB^{hi}$ T cells) resulted in a disease manifested by chronic nonbloody diarrhea and wasting. Transfer of the entire $CD4^+$ T cell subset did not result in disease and neither did the transfer of the reciprocal subset expressing low levels of the CD45Rb molecule ($CD4^+$ $CD45RB^{hi}$). Diarrhea and weight loss developed within weeks of the transfer. The disease was progressive unremitting and led to the death of the animal. Histopathologic changes in the recipients, which appeared very similar to other models of colitis, were limited to the intestine, mainly the colon, which was markedly thickened due to hyperplasia (Elson et al., 1995).

Apoptosis in colitis was investigated by Bregenholt, Petersen, and Claesson (2001) on a different adoptive transfer model by transplantation of SCID mice with purified $CD4^+$ T cells from immune-competent donors, resulting in the development of IBD in these mice. They have showed that colonic lamina propria was a major site for the presence of apoptosis in $CD4^+$ T cells in their model. Lamina propria $CD4^+$ T cells from SCID mice with colitis expressed both the Fas and Fas ligand (FasL) and stained for intracellular TNF-α when activated in vitro. Inasmuch as the two major mechanisms that existed for the induction of apoptosis were well documented to be either the Fas–FasL system or the TNF-α system, Bregenholt et al. showed that the lamina propria $CD4^+$ T cells are cytotoxic by a FasL-dependent mechanism but not via release of soluble TNF-α. Being indentified as an important apoptosis-inducing mechanism in pathogenic T cells in the inflamed colonic mucosa, targeting the Fas–FasL system in therapeutic strategies and hence controlling the accumulation of pathogenic T cells in the lamina propria of colitis patients, might be of certain benefit in future treatments of human IBD (Bregenholt, Petersen, & Claesson, 2001).

Furthermore, another adoptive transfer model was used by Wirtz, Becker, Blumberg, Galle, and Neurath (2002) to attempt a mucosal gene transfer using adenoviral vectors producing IL-18 antisense RNA to achieve selective down-regulation of IL-18 mRNA and protein levels in the experimental colitis colonic mucosa. Highly elevated IL-18 levels have been detected in lamina propria mononuclear cells and colon epithelial cells of subjects suffering from CD. Furthermore, it has been previously suggested that IL-18 was a potent regulatory factor for both proliferation and Th1 cytokine production by lamina propria T-lymphocytes in this disease. In this model, SCID mice were reconstituted with $CD45RB^{hi}$ or $CD62L^+CD4^+$ T cells leading to a chronic transmural colitis in almost 80% of the recipient mice 6–12 weeks after the T cell transfer. Large amounts of IFN-γ were produced in this model, and the important regulatory function of IL-18 by attempting to block IL-18 indicated a potential therapeutic relevance. Wirtz et al.

administrated IL-18 antisense RNA produced by an adenovirus and found that it was capable of suppressing IL-18 production by binding to IL-18 mRNA. Their endoscopic and histologic findings showed suppression of mucosal IFN-γ production and colitis activity in the thus treated mice (Wirtz et al., 2002). Although this gene therapy approach might be an attractive one for the manipulation of CD and despite recent advances in the understanding of mucosal immunology, effective manipulation of the gut immune system remained a challenging and controversial issue.

2.6. Less frequently used models of IBD

In addition to the 20 or more aforementioned animal models of IBD, there is a number of other models that do not fit any of the categories and are less frequently used. Such a category includes the germ-free mice model which exhibits some characteristics similar to human UC when injected with microorganisms isolated from the feces of genetically identical mice. In addition, a similar model, with inflammation and infiltration of immune cells as well as secretion of inflammatory cytokines, results from the oral administration of *Citrobacter rodentium* to young mice (Higgins et al., 1999). Finally, a combination of gamma irradiation and MHC class II deficiency in mice results in 100% penetrance of colitis. This is relatively a novel model of radiation induced colitis (Eliakim et al., 1999).

3. Conclusion

Based on the most recent medical literature and on our clinical and basic research experience, it appears that animal models of IBD have had a significant impact on the understanding of the disease and have contributed a great deal to the identification of new therapeutic agents. It is becoming clear that the most difficult decision in IBD may be to establish the correct diagnosis and find the most appropriate therapy. In this context, it is important to know whether UC and CD constitute a continuum of one clinical entity (i.e., inflammation) with a wide range of manifestations depending on immunologic and environmental parameters. While none of the models described are exact replicates of a human disease, coordination of multicenter studies has led to significant progress in this area. Differences between models may reflect the different subgroups of patients with IBD. Interestingly, it would appear that the proliferation of animal models of IBD has not only fuelled the detection of novel therapeutic agents but has also contributed to the understanding of the pathogenesis of IBD.

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